

Research Note

Radiation Sensitization and Postirradiation Proliferation of *Listeria monocytogenes* on Ready-to-Eat Deli Meat in the Presence of Pectin-Nisin Films[†]

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ABSTRACT

In this study, the ability of pectin-nisin films in combination with ionizing radiation to eliminate *Listeria monocytogenes* and inhibit its postirradiation proliferation was evaluated. Pectin films containing 0.025% nisin were made by extrusion. The surface of a ready-to-eat turkey meat sample was inoculated with *L. monocytogenes* at 10⁶ CFU/cm² and covered with a piece of pectin-nisin film. The samples were vacuum packaged and irradiated at 0, 1, and 2 kGy. The treated samples were stored at 10°C and withdrawn at 0, 1, 2, 4, and 8 weeks for microbial analysis. Reductions in *L. monocytogenes* viability of 1.42, 1.56, 2.85, 3.78, and 5.36 log CFU/cm² were achieved for the treatments of 1 kGy, pectin-nisin film, 2 kGy, 1 kGy plus pectin-nisin film, and 2 kGy plus pectin-nisin film, respectively. The greatest reduction (5.5 log CFU/cm²) was observed at 1 week for the 2 kGy plus pectin-nisin film treatment, suggesting that nisin was further released from the film to the surface of meat samples. Pectin-nisin films used in this study did not prevent but did significantly slow (*P* < 0.05) the proliferation of the *L. monocytogenes* cells that survived irradiation during 8 weeks of storage at 10°C. These data indicate the potential use of pectin-nisin films alone or in combination with ionizing radiation for preventing listeriosis due to postprocessing contamination of ready-to-eat meat products.

Listeria monocytogenes is a frequent postprocessing contaminant on ready-to-eat (RTE) delicatessen products. Infection caused by *L. monocytogenes* contributes to an estimated 2,500 serious illnesses and 500 deaths in the United States each year (4). Since 1980, this microorganism has caused several major outbreaks of human foodborne listeriosis in multiple states and prompted large-scale recalls of RTE meats (3–5). In a subsequent risk assessment, food contact surfaces throughout the manufacture and retail chain were cited as major points for potential *Listeria* contamination (32).

Ionizing radiation is a safe and effective method for inactivating foodborne pathogens on a variety of food products, including RTE meats, and a petition to allow irradiation of RTE foods is currently being evaluated by the U.S. Food and Drug Administration (8, 11, 13, 17, 27, 28). However, ionizing radiation at high doses can induce lipid oxidation, loss of color, or off-odors in meat products (1, 6, 21, 27). An alternative to treatment with high doses is to increase the radiation sensitivity of the target pathogens so that lower radiation doses can be used and product quality changes will be reduced. One approach to lowering the

required radiation dose is the use of antimicrobial packaging materials.

Nisin is an antimicrobial peptide that has been added to primary packaging materials to inhibit bacterial growth in foods, including meat products (10, 18–20, 24–26). Pectin is a water-soluble hygroscopic polymer that has been used as a thickening, coating, and encapsulating material. It also can be used as a vehicle to carry and deliver a variety of bioactive substances (15). However, few studies have been conducted to determine the effect of pectin-nisin films plus irradiation on *L. monocytogenes* in RTE meats.

RTE meat products subjected to temperature abuse (10°C) may contain *L. monocytogenes* at levels higher than 10⁴ CFU/g (12, 23, 33). Therefore, high cell density and 10°C were the conditions used in this study. The purpose of this study was to investigate the feasibility of using ionizing radiation to inactivate pathogenic *L. monocytogenes* strains inoculated onto turkey deli meat in the presence of pectin-nisin films. The ability of the pathogen to proliferate after sublethal doses of ionizing radiation under conditions of mild temperature abuse (10°C) for 8 weeks also was investigated.

MATERIALS AND METHODS

Meat sample. Presliced turkey deli meat without preservatives was purchased from a local vendor; the thickness was approximately 4 mm. Deli meat slices were punched into 40-mm-diameter disks, and meat samples were vacuum packaged and ir-

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[†] Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

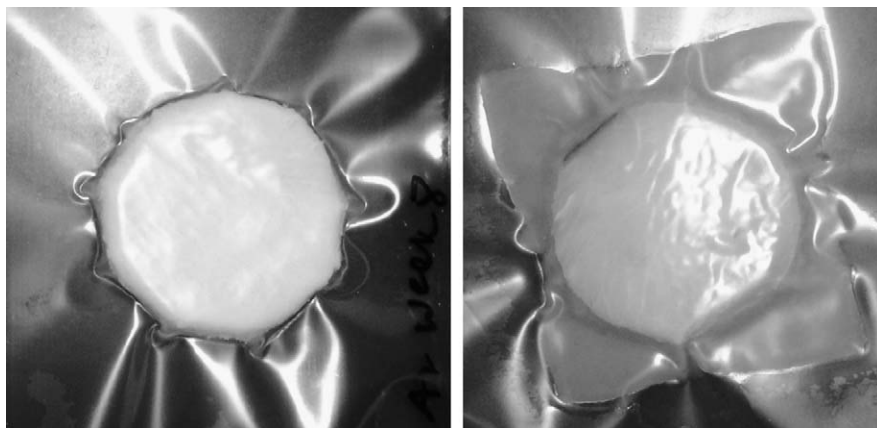


FIGURE 1. Vacuum-packaged RTE meat samples without pectin film (left) and with pectin film (right).

radiated at 10 kGy at -20°C to eliminate background microflora (Fig. 1). After irradiation, samples were maintained in a freezer (-20°C). Before the experiments, the meat samples were thawed overnight in a refrigerator (4°C).

Film preparation. Films were made by extrusion (16). Composite films were prepared from pectin, proteins of chicken egg albumin (EA) or porcine skin gelatin (SG), glycerol, and Nisaplin (Danisco-Cultor, Kansas City, KS), which contains 2.5% nisin salts and milk solids. The composition of the film was 50:19:30:1 (polysaccharide:protein:plasticizer:bacteriocin, wt/wt), and the film was extruded with a Werner-Pfleiderer ZSK30 co-rotating twin-screw extruder (Coperion Corporation, Ramsey, NJ) in the presence of water (25 to 30% total mass). Before feeding into the barrel, components were mixed with a heavy-duty blender. Compounding was performed at a screw speed of 250 rpm. The residence time was approximately 90 s. The performance temperature was kept below 120°C during extrusion and 85°C at the die. Strands obtained were ground with a Fritch mill (Kilion Extruders, Inc., Cedar Grove, NJ). The particles were then fed into a Brabender single-screw extruder equipped with a hangar-type die with a width of 5.0 cm and thickness of 0.5 mm for film production. The pectin films were cut into squares (45 by 45 mm) with an area large enough to cover the meat samples.

Mechanical properties of films. Tensile properties of the composite films were evaluated with an Instron model 1122 mechanical property testing machine (Instron Corp., Norwood, MA) with a 1 kN load cell according to ASTM D412-68 (15). The gauge length was 33 mm, and the strain rate was 50 mm/min. Mechanical properties evaluated were tensile strength, modulus, and elongation. All samples were conditioned for more than 48 h at 23°C and 50% relative humidity.

Bacterial strains. Four *L. monocytogenes* strains (H7762 serotype 4b, H7764 serotype 4b, F4249 serotype 1/2a, and F4561 serotype 1/2a) were obtained from the Centers for Disease Control and Prevention (Atlanta, GA). The strains were propagated on tryptic soy agar (Difco, Becton Dickinson, Sparks, MD) at 37°C and maintained at 0 to 4°C until use.

Inoculation. Each *L. monocytogenes* strain was cultured independently in 25 ml of tryptic soy broth (Difco, Becton Dickinson) at 37°C and shaken at 150 rpm for 18 h. The cultures were then combined, and the cocktail was sedimented by centrifugation (5,000 rpm for 5 min). The *L. monocytogenes* cocktail was then concentrated by resuspension in 12 ml of Butterfield's phosphate buffer (BPB; Applied Research Institute, Newtown, CT). Turkey slices (approximately 12.5 cm^2 each) were placed on a sterilized

cutting board in a biohood and surface inoculated on a single side with 0.1 ml of the *L. monocytogenes* cocktail. The inoculum was then spread over the product surfaces using sterile wet (BPB) cotton swabs. The inoculated cell density on sample surfaces was approximately 6 log CFU/cm². One piece of pectin film (45 by 45 mm) was put on top of each of inoculated meat slice.

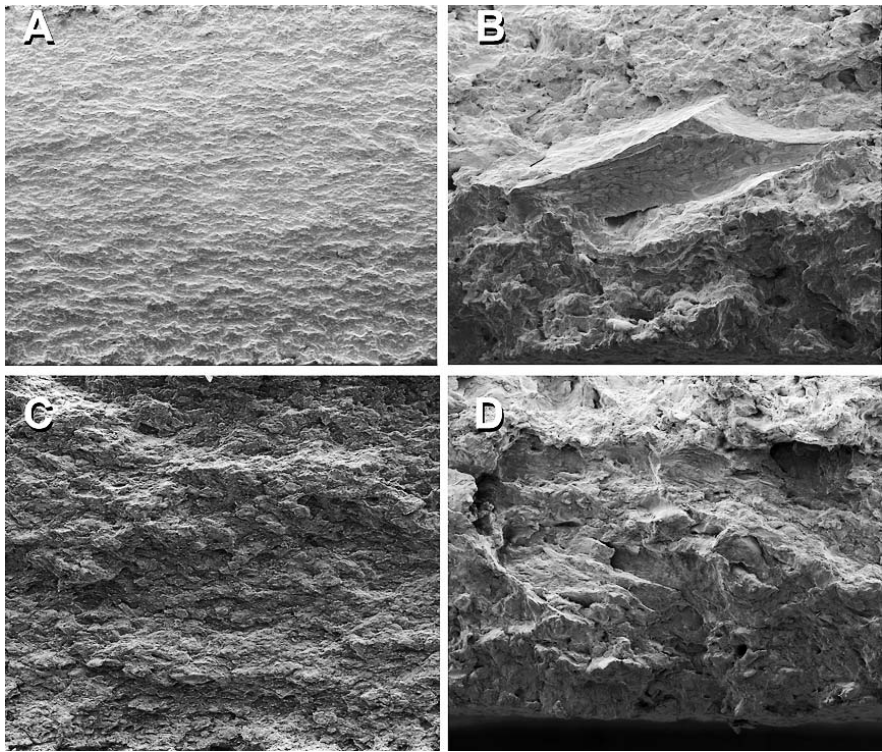
Assembled meat samples were packaged into a vacuum pouch (152.4 [length] by 203.2 [width] by 0.08 [thickness] mm, Polynylon; Uline, Inc., Waukegan, IL) and vacuum sealed at 700 mmHg after the internal air was evacuated. Meat samples without film also were vacuum packaged.

Gamma radiation. A self-contained ^{137}Cs radiation source (Lockheed Georgia Company, Marietta, GA) was used for all exposures. The dose rate (0.095 kGy/min) was verified using dosimeters obtained from the National Institute of Standards and Technology (Gaithersburg, MD). The temperature during irradiation was maintained at $4.0 \pm 1^{\circ}\text{C}$ by the gas phase of a liquid nitrogen source that was introduced directly into the top of the sample chamber. The temperature was monitored using two thermocouples placed on the side of the sample bags. The absorbed dose was recorded with 5-mm alanine pellet dosimeters that were attached to the sides of the sample bags, and the data were recovered with an EMS 104 EPR analyzer (Bruker, Billerica, MA). Radiation doses of 0, 1, and 2 kGy were used in this study. After irradiation, the samples were stored at 10°C for 8 weeks to simulate mild temperature abuse.

Microbial analysis. Following irradiation and during storage, three bags per treatment were removed at each sampling time, and the samples were assayed for *L. monocytogenes* using standard pour-plate procedures. Fifty milliliters of sterile BPB was added to a bag that contained a sample, and the sample was mixed by stomaching for 90 s. The samples were then serially diluted in BPB (10-fold dilutions), and 1 ml of a diluted sample was pour plated using *Listeria*-specific Palcam Agar (Difco, Becton Dickinson) with Palcam selective supplement (Oxoid, Basingstoke, UK). Three 1-ml aliquots were plated per dilution. The plates were then incubated for approximately 48 h at 37° before enumeration.

SEM. For determining the surface structure of pectin films before they were used on meat samples, the composite films were evaluated by scanning electron microscopy (SEM) as reported previously (14, 16). Specimens of films were dehydrated by immersion in gradient ethanol and then critically freeze-dried. The dried specimens were fractured manually with the edge of a cooled scalpel blade. Fragments were mounted to specimen stubs with adhesive, and the edge of the specimen was painted with colloidal silver adhesive. The specimens were then sputtered with a thin

FIGURE 2. SEM images of composite films of pectin plus egg albumin (A and C) or porcine skin gelatin (B and D). Nisaplin was included in films of C and D. Field width, 0.5 mm.



layer of gold and examined in a Quanta 200 FEG microscope (FEI Co., Hillsboro, OR) operated in high vacuum, secondary electron imaging mode. Images were collected at magnifications of $\times 5,000$ and $\times 250,000$.

SEM was also used to determine the surface characteristics of meat samples covered with pectin films. Slices of the turkey meat samples with pectin film were peeled apart and immersed in 20 ml of a 2.5% glutaraldehyde plus 0.1 M imidazole buffer solution (pH 7.2). After fixation, slices were washed in imidazole buffer and dehydrated by exchange with 20 ml of graded ethanol solutions: 50, 80, and 100%. Slices were critical point dried with liquid CO₂ in a DCP-1 critical point dryer (Denton Vacuum, Inc., Moorestown, NJ). The base of each slice was glued with Duco cement (ITW Performance Polymers, Riviera, FL) to specimen stubs, and the specimen was sputter coated with a thin layer of gold using a Scancoat Six Sputter Coater (BOC Edwards, Wilmington, MA) for 180 s. Digital images of topographical features of the turkey samples were collected using a Quanta 200 FEG environmental scanning electron microscope (FEI Co.) operated in the high vacuum, secondary electron imaging mode at an accelerating voltage of 10 kV.

TABLE 1. Tensile properties of pectin-protein-glycerol composite films^a

Film type	Tensile strength (MPa)	Young's modulus (GPa)	Elongation at break (%)
Pectin-EA	20.3 \pm 0.6	0.222 \pm 0.012	29.3 \pm 0.6
Pectin-EA with Nisaplin	12.8 \pm 0.8	0.173 \pm 0.016	19.7 \pm 1.7
Pectin-SG	7.1 \pm 0.6	0.067 \pm 0.005	27.4 \pm 4.2
Pectin-SG with Nisaplin	4.1 \pm 0.7	0.047 \pm 0.005	21.6 \pm 4.2

^a All samples were plasticized with 30% glycerol and water.

Statistical analysis. All experiments were conducted in duplicate on different days, and triplicate samples were analyzed at each sampling time. For mechanical testing, all measurements were performed on five samples. Data are expressed as the mean \pm standard deviation. All data were analyzed with an analysis of variance using SAS version 9.1 software (SAS Institute, Cary, NC). Duncan's multiple range tests were used to determine the significance of the differences in mean values. Unless stated otherwise, significance was determined at the 5% level.

RESULTS AND DISCUSSION

Physical properties of films. Micrographs of pectin-EA and pectin-SG composite films are shown in Figure 2. SEM revealed that the frozen-fractured faces of pectin-EA films without Nisaplin were relatively smooth in comparison with pectin-SG films. More irregular particles were unevenly distributed in the pectin-SG films, resulting in a small holes and clefs. The inclusion of Nisaplin into the pectin composite films resulted in a rough and dense surface. This roughness was more obvious for the pectin-SG films than for the pectin-EA films. More dense microscale rods and holes were evident on the pectin-SG films.

Mechanical properties of pectin films with or without 1% Nisaplin are listed in Table 1. Pectin-EA films had higher values for tensile strength, Young's modulus, and elongation at break than did pectin-SG films, and pectin films with Nisaplin had lower values for all three measures. These results are consistent with the findings of the structural analysis by SEM, i.e., an uneven structure was always not as strong as an even structure of similar composition. The milk solids and salts in Nisaplin may contribute to the weakness of these pectin-nisin films. The addition of Nisaplin to films had no effect on the film thickness (0.4 mm) as compared with the film without nisin (data not shown).

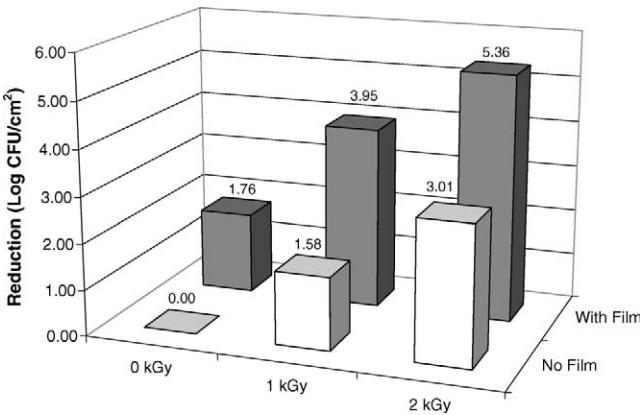


FIGURE 3. Effects of irradiation and pectin-nisin film on the reduction of *L. monocytogenes* on vacuum-packaged RTE turkey meat.

Our preliminary tests indicated that there was no significant difference between the two films in antimicrobial activity against *L. monocytogenes*; therefore, pectin-EA films, which had better mechanical properties, were selected for the following combination study.

Radiation sensitization of *L. monocytogenes*. The radiation sensitization of *L. monocytogenes* inoculated onto RTE turkey meat in the presence or absence of pectin-nisin films is shown in Figure 3. The initial inoculated levels of *Listeria* were approximately 6 log CFU/cm². Irradiation treatments of 1 and 2 kGy resulted in a 1.58- to 3.01-log reduction of *Listeria* cells, respectively. Pectin-nisin film treatment reduced *Listeria* by 1.76 log CFU/cm² without irradiation. However, the combination of irradiation with pectin-nisin film resulted in a 3.95-log reduction at 1 kGy and a 5.35-log reduction at 2 kGy; thus, an extra 0.6-log reduction was achieved by the combination treatment compared with the use of each individual treatment alone. These data indicate a synergistic effect on *Listeria* viability on the surface of RTE turkey meat.

In previous work (27), the reported *D*₁₀-value (the radiation dose required to achieve a 90% reduction in viable microorganisms) for *L. monocytogenes* inoculated onto deli turkey meat was 0.65 kGy, i.e., approximately 1.54- and 3.08-log reductions for the 1- and 2-kGy treatments, respectively. These findings are similar to those for irradiation

alone in the present study. Dawson and coworkers (10) also reported a 1-log reduction in *L. monocytogenes* populations on bologna packaged with soy films containing 5% Nisaplin and 8% lauric acid.

In the present study, treatment with pectin-nisin film without irradiation resulted in greater reduction in *L. monocytogenes* than did the 1-kGy treatment alone, and pectin-nisin film plus 1-kGy treatment resulted in greater reduction than did the 2-kGy treatment alone. These data indicate that (i) the pectin-nisin film can replace 1-kGy irradiation; (ii) the pectin-nisin film plus 1-kGy irradiation treatment can lower the radiation dose required to obtain similar microbial reduction achieved with 2 kGy irradiation alone; and (iii) greater pathogen reduction can be achieved with same dose (2 kGy) plus pectin-nisin film. Sommers and Thayer (30) found that a radiation dose of 2.45 to 3.55 kGy was needed to reduce *L. monocytogenes* levels by 5 log CFU on RTE meats, whereas in the present study the addition of pectin-nisin film to 2-kGy irradiation achieved a 5.35-log reduction of *L. monocytogenes*.

Postirradiation proliferation of *L. monocytogenes*. The effect of different treatments on vacuum-packaged RTE turkey meat inoculated with *L. monocytogenes* and stored at 10°C is shown in Table 2. *L. monocytogenes* on control meat samples were able to proliferate easily during the 8-week storage period (Table 2). The viability of *L. monocytogenes* applied to the surfaces of RTE turkey was not affected by pectin films without nisin. The pathogen population was not significantly different from that on the meat samples without film, indicating that pectin film itself did not have any antimicrobial activity against *Listeria* on the meat surface.

L. monocytogenes on RTE meat irradiated at 1 and 2 kGy was able to proliferate during storage at 10°C (Table 2). The pathogen population increased to 6.38 and 5.39 CFU/cm² by the end of the 8-week storage period, respectively. Sommers et al. (29) reported that *L. monocytogenes* on bologna irradiated at 1.5 kGy was able to recover quickly and reach a density of 6 to 8 log CFU/g within 4 weeks at 9°C. Thayer et al. (31) also found significant growth of *L. monocytogenes* on irradiated (1 and 2 kGy) cooked turkey breast meat stored at 7°C for 3 weeks.

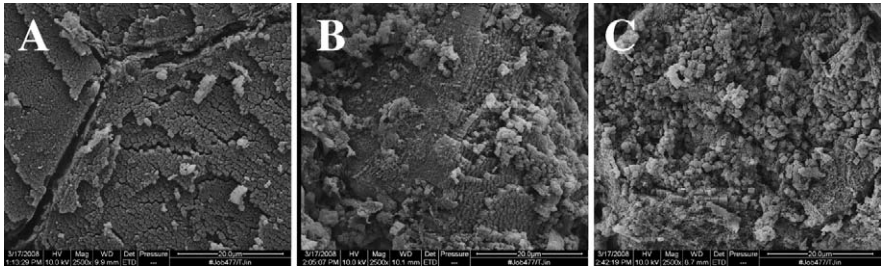
Ionizing radiation combined with pectin-nisin films can

TABLE 2. Postirradiation proliferation of *L. monocytogenes* on vacuum-packaged RTE turkey meats during 8 weeks of storage under conditions of mild temperature abuse (10°C)

Treatment	<i>L. monocytogenes</i> (log CFU/cm ²) at storage week ^a :				
	0	1	2	4	8
Control	5.98 ± 0.04 A	7.36 ± 0.09 A	7.61 ± 0.08 A	7.82 ± 0.07 A	8.30 ± 0.10 A
Pectin film without nisin	5.92 ± 0.12 A	7.44 ± 0.13 A	7.74 ± 0.09 A	7.77 ± 0.12 A	8.43 ± 0.09 A
Pectin-nisin film	4.22 ± 0.08 C	3.41 ± 0.04 C	4.65 ± 0.09 C	5.36 ± 0.08 C	5.63 ± 0.07 C
1 kGy	4.40 ± 0.07 B	4.56 ± 0.03 B	5.32 ± 0.05 B	5.86 ± 0.03 B	6.38 ± 0.08 B
2 kGy	2.97 ± 0.03 D	3.01 ± 0.06 D	4.13 ± 0.07 D	4.84 ± 0.05 D	5.39 ± 0.11 C
1 kGy + pectin-nisin film	2.03 ± 0.05 E	1.77 ± 0.07 E	3.16 ± 0.03 E	4.71 ± 0.09 D	5.59 ± 0.12 C
2 kGy + pectin-nisin film	0.63 ± 0.04 F	0.51 ± 0.05 F	1.18 ± 0.06 F	3.34 ± 0.07 E	4.04 ± 0.08 D

^a Values are means ± standard deviation. Within the same column, means with different letters are significantly different (*P* ≤ 0.05).

FIGURE 4. SEM images of the surface of meat samples: control (A); treated with pectin-nisin film, week 0 of storage (B); treated with pectin-nisin film, week 1 of storage (C).



significantly delay the proliferation of *Listeria*, as shown in Table 2. During the first week of storage at 10°C, no growth of *Listeria* was observed in the samples treated at 1 or 2 kGy, and even more reduction of *Listeria* was achieved for those samples treated with the pectin-nisin films or their combinations, suggesting that nisin was further released from films to the meat surface at week 1. Figure 4 proved SEM images for the surfaces of meat samples: control (Fig. 4A), samples treated with pectin-nisin film at week 0 (Fig. 4B), and samples treated with pectin-nisin film at week 1 (Fig. 4C). These SEM images revealed fine particles spread on the surface of meat samples treated with pectin-nisin film and stored for 1 week. The moisture in the meat allowed the particles containing nisin to be released from the pectin-nisin films to the meat surface and provide extended antilisterial activity. Natrajan and Sheldon (22) reported that the presence of moisture at the meat surface increased the efficacy of nisin incorporated into protein- and polysaccharide-based films.

At weeks 1 and 2, there were significant differences ($P < 0.05$) in *Listeria* populations among all the treatments. The samples treated with 2 kGy plus pectin-nisin film had the lowest cell counts followed by samples treated with 1 kGy plus pectin-nisin film, 2 kGy, pectin-nisin film, and 1 kGy.

Although *Listeria* cells gradually recovered and slowly grew during storage at 10°C, all treated meat samples at week 4 had cells densities lower than the inoculated cell density and significantly lower ($P < 0.05$) than that of the control sample. There was no significant difference in *Listeria* population ($P > 0.05$) between samples in the pectin-nisin plus 1 kGy treatment group and those in the 2 kGy treatment group at week 4.

The combination of irradiation and pectin-nisin film resulted in significantly lower *Listeria* populations throughout the 8-week storage period ($P < 0.05$) than were found when individual treatments applied. By the end of storage, the *Listeria* populations in the sample treated with 2 kGy plus pectin-nisin film was only 4 log CFU/cm², which was 2 log CFU/cm² less than the initial inoculated cell density and 4.3 log CFU/cm² less than the control. At the end of 8 weeks of storage, there were no significant differences in *Listeria* populations ($P > 0.05$) among the samples treated with 1 kGy plus pectin-nisin film, pectin-nisin film, and 2 kGy.

Listeria population in all samples with pectin-nisin films tended to increase after 1 week at 10°C. A variety of factors can reduce the effectiveness of nisin in a meat system. Nisin probably is inactivated by some meat compo-

nents, which might decrease the diffusion rate of nisin from the pectin-nisin film to the surface of the meat. In other meat products, nisin effectiveness was reduced by food characteristics such as pH, high fat content, particle size, and nonuniform distribution of nisin in food (2, 9).

The pectin-nisin films used in this study slowed but not prevent the proliferation of *L. monocytogenes* cells that survived irradiation. The differences in cell populations between irradiated samples with and those without pectin-nisin film (2 kGy versus 2 kGy plus pectin-nisin film and 1 kGy versus 1 kGy plus pectin-nisin film) decreased after 2 weeks of storage, further indicating that nisin released from film might lose activity. Increasing the nisin concentration in films may help to compensate for the loss of antilisterial activity during extended storage; this possibility should be evaluated.

Chen et al. (7) noted that low numbers of *L. monocytogenes* equals low health risk, and Gombas et al. (12) noted that the majority of contaminated RTE meats have *L. monocytogenes* populations lower than 10³ CFU/g. In the present study, irradiation at 1 or 2 kGy, pectin-nisin film, and the combination of irradiation and film significantly reduced *Listeria* populations on RTE meat ($P < 0.05$). The pectin-nisin film used in this study could replace the 1-kGy irradiation treatment or could be combined with irradiation to achieve greater *Listeria* reduction. This study showed for the first time the synergistic effect of the combination of irradiation treatment plus pectin-nisin film. The combined treatment of 2 kGy plus pectin-nisin film resulted in the greatest reduction of *Listeria* populations and significantly slowed *Listeria* growth ($P < 0.05$) during the 8-week storage period. Data from this study indicate the potential use of ionizing radiation in combination with pectin-nisin antimicrobial packaging for preventing listeriosis due to post-processing contamination or for preventing *L. monocytogenes* growth in accidentally recontaminated packages of irradiated RTE meats.

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